SCREENING FOR MICROSOMAL FORMATION OF AMIDES FROM CERTAIN BENZYLIC AMINES

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Amides are metabolites arising from certain secondary aromatic amines. Metabolic studies have shown amide formation from certain substituted benzyl anilines. We have extended these observations to other substrates bearing an N-benzyl moiety i.e. N-benzyl carbazole and N-benzyl pyrrolidine together with a variety of substituted benzyl anilines.

We wish to establish the mechanism(s) involved and the structure activity relationship for amide formation. Benzylic amines with different chemical properties were used to modify steric, electronic and pKa effects.

The results indicate that the corresponding nitrone is not an intermediate in the metabolic conversion of N-benzyl amines to the N-benzoyl derivatives, although they may be involved in the chemical production of amides produced during analysis. N-benzoyl derivatives were not formed during metabolic oxidation of N-benzyl carbazole or N-benzyl pyrrolidine indicating that pKa is not the controlling factor in metabolic amide formation. Steric hindrance around the benzylic carbon inhibited the process. We conclude that the metabolic formation of amides from N-benzyl amines probably involves an initial metabolic attack at the benzylic carbon atoms. The results indicate that both steric effects and pKa influence the formation of amides from N-benzyl aniline substrates.

DISPOSITION AND METABOLISM OF YM175, A NEW POTENT BONE RESORPTION INHIBITOR, IN RATS

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The disposition and metabolism of YM175, a new potent bone resorption inhibitor with bisphosphonate structure, have been studied in rats.

After oral administrations of YM175 in the dose range of 3-30 mg/kg, plasma concentrations of intact drug reached maximum levels within 0.5 h and decreased with half-lives of 0.32-0.72 h. Bioavailabilities were 0.74-2.64 %, increasing with increment of dose. After intravenous administration of ^{14}C-YM175 at 0.3 mg/kg, radioactivity was selectively uptaken in bone and slowly decreased with half-lives of 200-300 days. Autoradiogram showed higher level of radioactivity at the metaphysis in humerus. Although the metaphysis extended with animal growth after dosing, the radioactivity remained in the initially incorporated site. The radioactivities excreted in urine for 168 hrs after intravenous and oral administration were 49 % and 2.8 %, respectively. The ratios of intact drug to the radioactivity in urine after intravenous and oral administration were 76-87 % and 16-28 %, respectively. These results show that the absorption rate of this drug is very low and there is quantitative difference in metabolism between these two dosage routes.

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XENOBIOTIC METABOLIZING ENZYME ACTIVITIES IN MONO- AND COCULTURES OF RAT LIVER PARENCHYMAL CELLS WITH RAT LIVER NONPARENCHYMAL EPITHELIAL CELLS OR MOUSE EMBRYO FIBROBLASTS

Dietmar Utesch, Karl Ludwig Platt, and Franz Oesch, Institute of Toxicology, University of Mainz, Mainz, FRG.

Rat liver parenchymal cells (PC) rapidly lose their differentiated functions under standard cell culture conditions (monoculture). Coculture of PC with rat liver nonparenchymal epithelial cells (NEC) or mouse embryo fibroblasts (C3H) was shown to stabilze some of these PC functions in vitro. In the present study we present data about the activities in a series of xenobiotic metabolizing enzymes in the 7 day-old PC mono- and coculture systems.

The results of these investigations are:

(1) Almost all investigated enzyme activities were better preserved in the coculture systems as compared to monocultures.

(2) However, not all parameters were stabilized to the same extent. This means that the profile of the activities of xenobiotic metabolizing enzymes has changed after 7 days in culture.

(3) At least in this investigation, PC were better stabilized in coculture with C3H as compared to NEC.

2028903404

SEX DIFFERENCES IN BENZO(A)PYRENE TOXICOKINETICS IN THE RAT: THE INFLUENCE OF CHRONIC ETHANOL PRETREATMENT

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We are currently investigating the effect of chronic ethanol consumption on the toxicokinetics and mutagenicity of benzo(a)pyrene [B(a)P] in rats. In rat liver microsomes, a sex difference was already described for B(a)P metabolism (1). The interaction with ethanol was also suggested to be sex differentiated (2).

Male and female rats were kept on an ethanol containig diet for six weeks (3). At the end of this treatment postmitochondrial and microsomal fractions of liver were isolated and assayed for B(a)P metabolism. Another group of rats were given 1 mmol/kg B(a)P in the last week of ethanol pretreatment, orally or intraperitoneally, and urine and faeces were collected and analysed for B(a)P and its metabolites.

Urine of female rats contained less mutagenic compounds than urine of males after oral as well as after i.p. B(a)P administration. In general, less 3-hydroxy-B(a)P and 9-hydroxy-B(a)P was excreted in females. In urine of i.p. treated female rats, the excretion of both metabolites was significantly lowered after ethanol pretreatment.

In the faecesextracts five metabolites could be identified: 9,10-dihydrodihydroxy-B(a)P, 4,5-dihydrodihydroxy-B(a)P, 7,8-dihydrodihydroxy-B(a)P, 9-hydroxy-B(a)P and 3-hydroxy-B(a)P, both in males and females. No significant difference between males and females was found in the faecal excretion of mutagenic compounds. However, males generally excreted more B(a)P metabolites than females. After oral administration higher amounts of metabolites were excreted in faeces than after i.p. administration in males and females. A significant lowering effect of ethanol pretreatment was measured on the excretion of 3-hydroxy-B(a)P in males and females after oral and i.p. treatment.

In the in vitro experiments the five B(a)P-metabolites were detected after incubation with male liver fractions. In females, in vitro metabolism was significantly lower than in male rats and only three metabolites were found. There was a significant lowering effect of the ethanol pretreatment on the formation of B(a)P metabolites in vitro.

In conclusion: In rats a pronounced sex difference occurs in B(a)P metabolism, both in vivo and in vitro. Ethanol pretreatment significantly impaires formation of B(a)P metabolites. This effect however does not seem to be sex differentiated.

Literature

1. Gurtoo and Parker (1977) Drug Metab. Disp. 5, 474-481
2. Seitz et al. (1981) Cancer Lett. 13, 97-102
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N-DEMETHYLATION OF THEOPHYLLINE BY THE AROCLOR 1254-INDUCED RAT LIVER MICROSOMES

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Theophylline (T) is regarded as a possible marker of MC family cytochrome P-450 *in vivo*. Recently the proofs have been obtained which evidence that P-450 of MC-family are not the sole metabolisers of T. Direct evidence have been obtained which that the principal metabolite of T *in vivo*,namely, 1,3-dimethyluric acid (1,3-DMU), reflect the activities of MC-inducible P-450IA2 together with constitutive P-450IIC6. In our experiments treatment of the animal with PB or MC results in a similar increase of the output of 1,3-DMU. Unlike PB, treatment with MC sufficiently increases also production of 3-methylxanthine (3-MX) and 1-MX. Treatment with Aroclor 1254 resulted in dramatic increase of production of 1,3-DMU and 1-MX. Of importance is the fact that the greate increase in production of 1-MX was not followed by concomitant increase in the output of 3-MX. The kinetics of 3-MX formation in the Aroclor microsomes was monophasic in the concentration range from 50 μM to 2 mM. Saturating concentration of T was 0.5 mM Km = 89 μM. The kinetics of 1-MX production was biphasic and coincident with those for 3-MX at low concentrations (Km = 95 μM). The rate of this reaction increased by 3-4 fold when the concenration was increased from 0.5 to 2 mM (Km = 2.5 mM). This results evidence the participation of more than one form of P-450 in 3-N-demethylation of T.

2028903405

ENANTIOSELECTIVE PHARMACOKINETICS OF PROPRANOLOL IN THE RAT : MARKED DIFFERENCES BETWEEN RAT AND MAN.

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In rats, plasma protein binding of R- and S-propranolol differs markedly while in humans this is not so. Therefore we evaluated whether there is marked enantioselectivity in the kinetics of propranolol in the rat after administration of the racemate.
After IV administration, for R-propranolol the AUC is two times higher than for S-propranolol ; clearance, volume of distribution and half-life are lower than for S-propranolol.
After oral administration, the difference in AUC between the two enantiomers is more pronounced (five times higher for R- than for S-propranolol). No difference in intrinsic clearance is observed.

The enantioselectivity in propranolol kinetics observed in the rat is in marked contrast with findings reported for humans in the literature. In humans, the differences between the enantiomers are less pronounced ; clearance and volume of distribution for R-propranolol are higher than for S-propranolol ; the intrinsic clearance shows no differences between the enantiomers.

The marked species differences are probably due to plasma protein binding differences.

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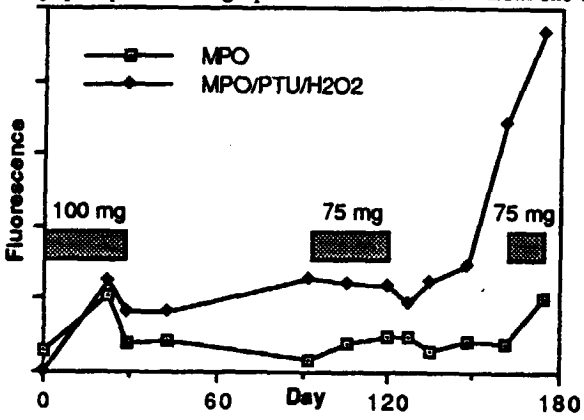
ANTIBODIES TO MODIFIED MYELOPEROXIDASE IN A FELINE MODEL OF DRUG-INDUCED AUTOIMMUNE DISEASE

Lisa Waldhauser and Jack Uetrecht, Faculty of Pharmacy, University of Toronto, Toronto, Canada, M5S 2S2

Propylthiouracil (PTU) is one of many drugs that cause hypersensitivity reactions such as agranulocytosis and drug-induced lupus. We have previously shown that PTU is oxidized by the myeloperoxidase (MPO)/H_2O_2 system of neutrophils to several reactive metabolites. These may haptenize proteins, such as MPO, and induce formation of antibodies against the native or modified protein. We investigated this possible mechanism in cats that developed PTU-induced autoimmune disease.

Two of five cats treated daily with oral PTU developed a syndrome of anorexia, anaemia, lymphadenopathy and elevated immunoglobulin levels during three courses of treatment. At the third challenge, both were sacrificed due to severe anaemia.

Serum was tested for anti-MPO antibodies by an ELISA using MPO incubated with PTU and H_2O_2 under conditions in which PTU is metabolized, as well as untreated enzyme. Binding was determined with alkaline phosphatase rabbit anti-cat IgG and methylumbelliferryl phosphate. The graph shows ELISA results from one of these cats.



The data shows increased recognition of modified MPO during the most acute illness without an increase in binding to the native enzyme. The presence of antibodies specifically directed against modified MPO suggests metabolism of the drug by MPO alters the enzyme in such a way as to induce antibody formation, and this process may be involved in development of disease. (Supported by Medical Research Council MA-9336).

DISPOSITION OF BROFAROMINE HYDROCHLORIDE (CGP 11305 A) IN THE BABOON.

Felix Waldmeier and Peter H. Degen.

CIBA-GEIGY Limited, Pharma Research and Development, K-136.2.84, 4002 Basel, Switzerland.

Brofaromine hydrochloride (B) is a new selective reversible MAO A-inhibitor currently undergoing clinical trials as an antidepressant drug. Its disposition was investigated in two male baboons after single 3 mg/kg doses of a 14C-labelled preparation. Blood, plasma, urine and faeces were analysed using radiometry, GLC and HPLC-radiometry.

After iv dose, B declined in plasma with a mean terminal half-life of 9 h, radioactivity with one of 20 h. Perorally dosed B was completely absorbed and peak plasma levels were observed at 8 h after dose. The plasma levels of B declined with a half-life of 13 h, radioactivity with one of 16 h. B accounted for about 17 % of the plasma radioactivity (AUC), conjugated B for 41 %. B was eliminated by extensive biotransformation, mainly by oxidative demethylation to CGP 35748, followed by glucuronidation and presumably sulfation. The resulting conjugates accounted for 19 % of the plasma radioactivity and 63 % of the radioactivity in urine. About 80 % of the dose was excreted within 2 days, excretion was almost complete after 7 days. At least 80 % of the dose was excreted renally. In conclusion, the kinetics, biotransformation and excretion of B in the baboon and the systemic exposure of the baboon to B were comparable to what was observed in man.

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ANTIBODIES TO MODIFIED MYELOPEROXIDASE IN A FELINE MODEL OF DRUG-INDUCED AUTOIMMUNE DISEASE

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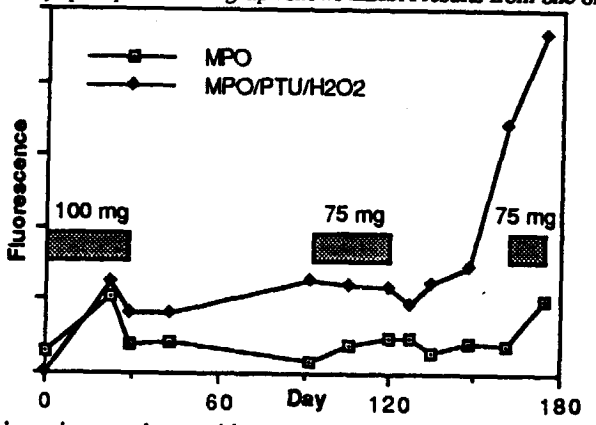

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METABOLISM OF ICI 118,630 BY ISOLATED PERFUSED LIVER AND KIDNEY

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Specificity and activity of peptidase activity varies fromtissue to tissue. In vivo studies withICI 118,630 revealed differences in metabolite patterns between plasma, urine and bile which could be attributed to tissue specific metabolism and excretion. Rat livers and kidney were perfused with Krebs-bicarbonate buffer containing washed bovine red cells. [^{14}C]-ICI 118,630 was added to the perfusion medium and timed samples of perfusate and bile/urine collected up to 240/120 min. Samples were assayed radiochemically and by reverse phase HPLC to establish metabolite patterns. Both organs were capable of metabolising the compound. In liver, clearance was rapid and a range of metabolites, including 1-8,1-9,1-7 and 3-10 were found in perfusate. Approx 30% of the 'dose' was eliminated in bile during the perfusion period. No parent compound was detectable, bile containing primarily 5-7,5-9 and 1-7. Clearance in kidney was rapid. Circulating metabolites indicated metabolism at both C and N terminii. The major urinary components were 5-8, 1-8 and 5-7.

ATTEMPTS TO STABILIZE THE CYTOCHROME P-450 DEPENDENT MONOOXYGENASE ACTIVITY IN PRIMARY CELL-CULTURE

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Primary cultures of hepatocytes potentially are an important model-system for studies of the biotransformation of xenobiotics but suffer from a rapid decay of cytochrome P-450 dependent phase-I-reactions. In the present study we have investigated whether this rapid loss of monooxygenase activity can be prevented by a) co-cultivation with rat liver epithelial cell clones, b) addition of different inducing agents, c) use of a perifusion system and d) changes of the extracellular matrix. Cultures were maintained for up to 10 days. Biphenyl and lonazolac were used as model substrates. Their metabolites were separated by HPLC with fluorescence detection. The decrease of the P-450 isozyme pattern is slowed down differently by co-cultivation with epithelial cells. Depending on the clone used, inducing agents, in particular PB, may nearly prevent the loss of phase-I-reactions in hepatocytes. There is no difference between conventional co-culture and co-cultures in a perifusion system, even after addition of inducing agents. Changing the extracellular matrix from collagen to matrigel had no stabilizing effect. These results indicate that co-cultivation in combination with inducing agents results in the best stabilization of the cytochrome P-450 system. Such cultures may be successfully used to study the metabolism of xenobiotics in vitro.

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CHARACTERIZATION OF PRIMARY METABOLIC PATHWAYS OF OMEPRAZOLE BY LIQUID CHROMATOGRAPHY – SEX DIFFERENCES IN THE RAT

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The selective inhibitor of the gastric proton pump, omeprazole (Figure 1), is extensively metabolised in several mammalian species, including man. The main urinary metabolite is formed through hydroxylation of the 5–methyl–pyridine group(I), other primary routes being the formation of the 5– and 6–hydroxybenzimidazole metabolites (II and III, respectively). Several studies performed in our laboratories in different species indicate that formation of I may be catalysed by the genetically polymorphic mephenytoin hydroxylase (Cyt P450IIC9) (in vivo in humans), while in vitro studies in rats have shown that II is formed via an unknown, but phenobarbital inducible P450 isoenzyme, and III of 3–methyl cholanthrene inducible isoenzymes (possibly of the P450IA family).

An HPLC method was developed to separate these and other metabolites of omeprazole in urine and plasma samples from animals and man. Chromatographic separation was improved after selective reduction of the sulfoxide functionality to the corresponding sulfide. The reduction also stabilized the compounds towards acid degradation and made enzymatic hydrolysis at pH 5 of potential conjugates of II and III possible. In this way the sums of the unconjugated and conjugated metabolites were obtained, facilitating true estimations of the relative importance of the different primary routes.

Analysis of urine samples from male and female rats revealed dose dependent sex differences in the excretion of these primary metabolites as well as in their subsequent conjugation: Male rats preferentially formed I irrespective of the amount of omeprazole administered, while female rats given low doses of omeprazole (0.3 µmol/kg to 10 µmol/kg), excreted fairly large dose fractions of II and III, most of which were conjugated. At a higher dose (400 µmol/kg) to female rats, the metabolic pattern was similar to that of male rats, metabolite I being the most abundant metabolite excreted.

Preliminary results from human samples will be presented.

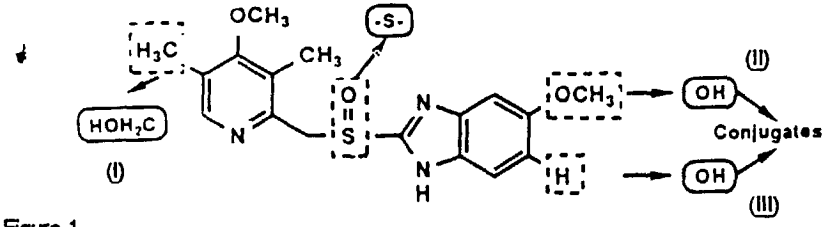

Figure 1

CYTOGENETIC EFFECTS (SCE) AND MUTAGENICITY OF 2-CHLORO-1,3-BUTADIENE (CHLOROPRENE)

G. Westphal, Elke Turner, H. Karels, H. Peter

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Chloroprene is used for the production of glues and as a monomer for the synthesis of chemical-resistant plastics (e. g. Neoprene™). The substance has not yet been classified by the IARC (International Agency for Research on Cancer) in regard to its carcinogenic potential. The close relationship of its chemical structure with the carcinogens vinyl chloride and 1,3-butadiene necessiate a thorough investigation of chloroprene.

Since pure chloroprene is unstable, the substance was purchased as a 50% solution in toluene and freshly distilled for each experiment. It was then preincubated with S. typhimurium TA 100 with or without addition of S9 Mix or GSH in gas tight screw cap vials.

In two parallel experiments, a differentiation between the cytotoxicity and the mutagenicity of chloroprene was performed. For the mutagenicity test, the substance was processed according to Ames. Cytotoxicity was determined by dilution of an aliquot of the stock culture with PBS buffer, preincubation with chloroprene as described above and plating on nutrient agar.

In light of the instability of chloroprene, a strict time regimen had to be followed for the interval between distillation of the substance and testing. The time dependent aging of chloroprene was controlled by gas chromatography. The poor mutagenicity of freshly prepared chloroprene did not differ in the samples with or without addition of S9 Mix. However, the mutagenic effect increased with the age of the distilled substance. Addition of glutathione (with and without S9 Mix) reduced the mutagenicity and the cytotoxicity of the substance.

In additional experiments, human lymphocytes were isolated and cultivated for SCE determination. After 24 hours of cultivation, chloroprene was added to the cultures and incubated for 48 hours. Processing was performed according to Perry & Wolff. A significant, dose dependent enhancement of the rate of SCE was found.

The experiments show that chloroprene is able to induce sister chromatid exchange (SCE) in human lymphocytes. Mutagenicity in the Ames-Test was not significant with freshly distilled chloroprene but increased with the aging of the instable chemical. Thus aging of glues containing chloroprene could lead to an increased health risk for the user.

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BIOACTIVATION AND COVALENT BINDING OF 7-HYDROXY-2-NITROFLUORENE IN THE RAT IN VIVO AND IN VITRO.

Frank C.J. Wierckx, Anja J.M. Timmers-Reker, John H.N. Meerman and Gerard J. Mulder. Division of Toxicology, Center for Bio-Pharmaceutical Sciences, University of Leiden, Leiden, The Netherlands.

Previous studies in our group indicated that oxidative metabolism is involved in the bioactivation of the air pollutant 2-nitrofluorene (2-NF) and therefore, we studied the bioactivation of an oxidatively formed metabolite: 7-hydroxy-2-nitrofluorene (7-OH-2NF). In vitro covalent binding to RNA of this metabolite is catalyzed by a rat liver microsomal fraction and is NADPH dependent. Its covalent binding could not be prevented by glutathione. Hydrolysis of the modified RNA by trifluoroacetic acid hydrolysis followed by HPLC analysis revealed one major adduct. This adduct did not coelute with any of the adducts formed from 2-NF in vivo. Therefore, formation of 7-OH-2-NF and subsequent microsomal bioactivation of this compound do not contribute to the bioactivation of 2-NF in vivo. When 7-OH-2-NF was administered intravenously to the rat in vivo, the compound became covalently bound to cellular macromolecules in the liver, kidney and lung.

Cytoprotection by iloprost against paracetamol toxicity in a genetically engineered cell line.

J.W. Wilson, A.R. Boobis, N. Battula[1], S.S.Thorgeirsson[1] & D.S. Davies. Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 ONN and [1]National Cancer Institute, NIH, Bethesda, MD, USA.

We have previously shown that paracetamol is toxic to the rat liver epithelial cell line, RLER52H-16 (R52), which has been transformed to express murine P4501A2. This toxicity is dependant upon the expression of the P450 gene by the cells (Wilson *et al* 1991). Treating cells previously exposed to 10 mM paracetamol for 2 hours with 1.25 mM N-acetyl-L-cysteine almost completely prevented the subsequent loss of plasma membrane integrity (as measured by lactate dehydrogenase leakage). This was associated with a restoration of the concentration of reduced glutathione (GSH) in the cells to control levels. The cytoprotective prostanoid iloprost, at 0.1 nM and 10 nM, added to the R52 cells following incubation of the cells with 10 mM paracetamol for 2 hours, and added again 3 and 6 hours later, reduced the loss of plasma membrane integrity by 15% (0.1 nM) and 30% (10 nM). Iloprost treatment had no effect on the concentration of GSH in paracetamol treated cells. Thus iloprost is cytoprotective in a cultured cell line. This should prove useful in future studies of the mechanism of cytoprotection.

1) Wilson J.W., Boobis A.R., Battula N., Thorgeirsson S.S. & Davies D.S. B.J.Pharm. (Proc.) (1991) - in press.

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Source: https://www.industrydocuments.ucsf.edu/docs/shdl0000

CHANGES IN THE COMPOSITION OF GLUTATHIONE S-TRANSFERASES IN RAT LIVER AFTER ORAL ADMINISTRATION OF BENZO[A]PYRENE

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Benzo[a]pyrene (B[a]P) is an ubiquitous environmental contaminant and a potent carcinogen in a variety of biological systems. Glutathione (GSH) S-transferases (EC 2.5.1.18) are a family of isoenzymes serving an important role in the biotransformation of many electrophilic compounds. They are known to be induced by many xenobiotics. In the present study, after oral administration of B[a]P, changes in the composition of hepatic GSH S-transferase subunits in rat were determined using a reverse-phase HPLC technique.

Oral administration of 50 mg/kg of B[a]P for 10 days resulted in an increase in the activity of cytosolic GSH S-transferases, but not of the microsomal GSH S-transferase. The cytosolic enzyme activities toward 1-chloro-2,4-dinitrobenzene and 1,2-epoxy-3-(p-nitrophenoxy)propane were increased by 1.9-fold and 1.6-fold, respectively, while little or no change was observed in the activities toward 1,2-dichloro-4-nitrobenzene and ethacrynic acid. When GSH S-transferase subunits were determined using a reverse-phase HPLC coupled with the S-hexyl affinity chromatography, subunit 1 was preferentially induced by the B[a]P treatment as compared with subunit 2, 3, and 4, thus explaining the substrate specrum of GSH S-transferases. This induction pattern was quite different from that in the rat treated with phenobarbital (80 mg/kg, i.p. for 4 days).

These data indicate that the elevation of the activity of GSH S-transferases after oral administration of B[a]P is mainly due to the selective expression of subunit 1, which may contribute to the detoxication of B[a]P and/or its metabolites.

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EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF A PHENOL SULFOTRANSFERASE cDNA, PST-1

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Sulfation is a main metabolic pathway of xenobiotics and endogenous chemicals. Lipophilic phenols are conjugated in rat livers to form the sulfates. This reaction is catalyzed by a family of cytosolic sulfotransferase (phenol or arylsulfotransferase). We have recently isolated three distinctive forms of sulfotransferase from male rat livers. Among them, a purified phenolsulphotransferase (PST-I), which showed 31 KDa on SDS-PAGE, catalyzed sulfation of various types of phenols, and was distingushed immunochemically from two other forms which catalyze sulfation of N-hydroxy-2-acetylaminofluorene. Using anti-PST-I antibodies, a cDNA (PST-1) was isolated and characterized to encode 291 amino acids with the molecular weight of 33,909. To further understand the characteristics of PST-1, we have expressed PST-1 in COS-1 cells and examined the catalytic properties. The cytosol expressed PST-1 catalyzed sulfation of various phenols including p-nitrophenol, α-naphthol and minoxidil.

Reference

Ozawa et al., Nucleic Acids Res., 18 4001 (1990).

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CHARACTERISTICS OF ORAL LYM-X-SORB/DRUG DELIVERY SYSTEMS

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Lysophosphatidyl choline (LPC) in the presence of monoglycerides (MG) and fatty acids (FA) results in eutectic molecular organizations (LYM-X-SORB) which entrap drugs within their inclusion space for enhanced oral bioavailability of drugs (D. Yesair, U.S. Patent 4,874,795).

Differential scanning calorimetry of defined mole ratios of these three components (LPC, MG & FA) yields a single melting point eutectic indicating a specific molecular organization. Viscosity of the individual components and their combinations indicates that LPC is the principal component for the interaction with MG and FA.

These eutectic formulations maintain their integrity in an aqueous environment. In the presence of aqueous bicarbonate and bile salts the sonicated LYM-X-SORB formulations yield 5-10 nm sized particles. The CMC of these aqueous micellular eutectic formulations is about 0.05 mM or two (2) orders of magnitude smaller than the CMC of sodium taurocholate. Inclusion of several drug classes, including peptides, in these systems will be presented.

These aqueous eutectic drug formulations are assimilated into the intestinal wall of rats within 15 minutes and eventually appear as plasma lipids/drugs over the next several hours. These eutectic LYM-X-SORB/DRUG systems markedly enhance the oral absorption of drugs via the lymphatics, yielding metabolite profiles that are similar to intravenously administered drugs in animals and humans. (BMP Ref. 33/ISX1ABST.DOC)

PHARMACOKINETICS AND METABOLISM OF DILTIAZEM IN HEALTHY VOLUNTEERS FOLLOWING A SINGLE ORAL DOSE

Pollen K.F. Yeung, Chris Prescott, Camille Haddad, Dorothy Marshall, Helen Tremayne, Carl McGregor, Michael A. Quilliam[1], and Terrence J. Montague. College of Pharmacy and Division of Cardiology, Dalhousie University and Victoria General Hospital, Halifax, Nova Scotia, Canada B3H 3J5. [1]Institute of Marine Biosciences, National Research Council, Halifax.

Plasma concentrations and urinary excretion of diltiazem (DTZ) and its metabolites were determined in 20 healthy volunteers (10 males and 10 females) given a single oral 90 mg dose of DTZ. DTZ and six of its metabolites which included N-monodesmethyl DTZ (MA), deacetyl DTZ (M1), deacetyl N-monodesmethyl DTZ (M2), deacetyl O-desmethyl DTZ (M4) and deacetyl DTZ N-oxide (M1NO) and deacetyl N,O-didesmethyl DTZ (M6), were determined by a sensitive and specific HPLC assay. The major metabolites measurable in the plasma of all the volunteers were MA, M1 and M2. The terminal half-lives ($t_{1/2}$) of M1 and M2 were considerably longer than those of DTZ and MA. Less than 5% of the dose was excreted as unchanged DTZ in the urine over the 24-hour period. The major urinary metabolite was MA, followed by M6, M2, and then M1. Except for the urinary excretion of M4 which was higher in the males, there were no statistically significant differences ($p > 0.05$) in any of the pharmacokinetic parameters between the males and the females. In addition, two other major urinary metabolites were isolated and characterized by ammonia chemical ionization mass spectrometry. They were tentatively identified as O-desmethyl DTZ (Mx) and N,O-didesmethyl DTZ (supported in part by Medical Research Council of Canada MA-10177 and Nova Scotia Heart Foundation).

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STUDY OF THE DEPOSITION OF A RADIOLABELLED COMPOUND FOLLOWING ADMINISTRATION AS AN AEROSOL TO LABORATORY RATS

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In support of the toxicological evaluation of a test compound following exposure via the inhalation route, the deposition of the compound in the lung and the subsequent systemic distribution can be studied using radiolabelled test compound. In this study, rats were exposed to atmospheres of the radiolabelled test material, generated from metered dose inhaler vials, using an ADG-Snout only exposure system. Metered dose inhaler vials were prepared to mimic the proposed marketable formulation, but containing carbon-14 labelled test material. Radiolabelled test material was used to validate the toxicology studies, by demonstrating that the experimental drug administration procedures used in the inhalation toxicology studies expose the rat lung to significant amounts of the test compound. The distribution patterns of radioactivity in the rat were examined immediately on cessation of the exposure, to estimate maximum lung concentrations of radioactivity, and at intervals up to 2 h post dose, to examine the depletion of radioactivity from the lungs and its subsequent systemic distribution. The tissue distribution was described by both whole body autoradiography and by quantitative methods. Results showed that the experimental system exposed the rat lung to test material. A mean of *ca* 2% of total body radioactivity was present in the lungs immediately after dosing. Clearance of radioactivity from the lungs and its subsequent distribution to organs, tissues and body fluids, in particular those of metabolism and elimination, was also demonstrated.

FAT AND MUSCLE ARE THE IMPORTANT DEPOT TISSUES IN RATS FOR ARTEETHER, A NEW ANTIMALARIAL DRUG DEVELOPED UNDER THE AUSPICES OF WORLD HEALTH ORGANIZATION

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Data on the distribution of drug to various tissues are obligatory for the new drug registration. To the present no report about arteether in this area has occurred. A liquid chromatography with electrochemical detector(LC-EC) for arteether distribution studies was developed in this institute last year. In this report the bioanalytical method and some preliminary results about arteether distribution are reported. Rats were intravenously given a single dose of 11.6 mg/kg arteether in fat emulsion, alternatively another rat was repeatedly given the same dose for 8 times with an interval of 10 min. The animals were sacrificed at 15 min after the last dose. The biosamples were homogenized and extracted with a solvent mixture of acetone and ethylacetate. The concentrated samples were analyzed by LC-EC, with the propyl analog of arteether as internal standard. Apparent recovery rates were 113% for fat and 98% for muscle. The concentration of arteether(ug/gm) for fat and muscle were found to be 18.4(S.D.10.7) and 1.4 (0.2) for the rats receiving the single dose, while 61.7 and 4.1 for the rat receiving the multiple doses. The total weight of fat and muscle in the body are approximately equal to 20% and 50% of the body weight respectively. The quantities of arteether in fat and muscle in the single dose group were thus amounted to 33.5(19.5)% and 6.2(0.9)% of the dose given; or according to the pharmacokinetic analysis, their sum was more than 90% of the arteether remaining in the body. In addition, some preliminary LC-MS data showed that the distribution profiles for arteether and its metabolites in fat and muscle were similar to those in plasma. The distribution to other tissues, including intestine, liver, kidney and brain is just now being studied. A physiologically-based pharmacokinetic model had been developed for artemether[1], a similar model will be applied to arteether. Reference [1]: Y.L.Zeng, Interspecies scaling for artemether, presented at IInd International ISSX Meeting, Kobe, Japan, 1988

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THE EFFECTS ON FREE RADICALS RELEASE AND LIPID PEROXIDATION BY TWO GERMANIUM COMPOUNDS

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Ge-401 is a new organic germanium compound developed in Shanghai Inst. of Building Materials. Ge-132(Carboxyethyl germanium sesquioxide) was originally synthesized in Japan. Their inhibitive effects on the growth of cancer had been reported. Free radicals play an important role in canceration. In order to elucidate the molecular mechanism of their pharmacological action, the results obtained in three kinds of experiments were reported. (1) Their effects on the generation of oxidative metabolites from respiratory burst in activated monocytes by 0.5um formyl-methionyl-leucyl-phenylalanine (FMLP) were studied. The superoxide released from the monocytes by FMLP was measured by means of cytochrome C(Cyt C) reduction. Ge-132 dose-dependently inhibited the Cyt C reduction. Complete inhibition was observed at the concentration of 2.06mM. The IC50 was estimated as 1.65mM. But no significant effect of Ge-401 was observed. (2) The rate of pyrogallol autoxidation also represented superoxide release. Thus the effects of two germanium compounds as well as vitamine C (Vit C) were compared. The IC50 on pyrogallol autoxidation by Ge-401, Ge-132 and Vit C were estimated as 0.029mM, 0.47mM and 0.04mM respectively. 0.056mM Ge-401 and 0.1mM Vit C inhibited pyrogallol autoxidation completely; but Ge-132 can not, even at a concentration as high as 0.983mM. (3) Ge-401 exerted a protective effect on lipid peroxidation of mice mitochondria. The malondialdehyde (MDA) content rose by 50% after ethanol administration. A dosage of 100mg/kg/ day of Ge-401 given ip for 10 days significantly inhibited the effect of ethanol on lipid peroxidation and lowered MDA content to normal level. Basing upon the above mentioned results we concluded that both Ge-401 and Ge-132 could influence the free radical metabolism under a certain condition. In addition, the pharmacokinetic and pharmacodynamic relationship is just under investigating.

INVOLVEMENT OF THE HUMAN HEPATIC CYTOCHROME P-450 IIIA ISOZYME IN THE BIOTRANSFORMATION OF VINDESINE AND VINBLASTINE

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The metabolism of vindesine (VDS) and vinblastine (VLB) was studied using human hepatic microsomal fractions. After incubation, both alkaloid were converted into one major metabolite which eluted just after the unchanged drug according to our chromatographic conditions. The formation of these metabolites follow Michaelis-Menten kinetics: the Michaelis constant (Km) and maximal velocity (Vmax) estimated by Lineweaver-Burk plot are 22.6 µM, 1.3 nmol/min/mg protein for VDS, and 7.0 µM, 1.2 nmol/min/mg protein for VLB. On the other hand, a number of compounds known to specifically inhibit the different human P-450 isoforms were tested for their effects on VDS and VLB biotransformation: ie, benzo(a)pyrene/theophylline (form IA), hexobarbital (IIC), debrisoquine/sparteine (IID), aniline (IIE), troleandomycin (TAO)/erythromycin (ER)/quinidine (QND) (IIIA). In the presence of these compounds, the formation of the major metabolites was significantly reduced only by the known inhibitors of the form IIIA. The inhibitory effects of TAO, QND and ER (expressed as percentage of control) were 99.0, 93.5, 66.8 % for VDS, and 73.3, 82.5, 56.8 % for VLB biotransformation respectively. Dixon plot demonstrated that these compounds competitively inhibited the biotransformation of VDS and VLB: the inhibition constants (Ki) of QND, TAO and ER on VDS biotrasformation were 3.6, 17.9 and 19.9 µM respectively and 2.7 and 22.5 µM for QND and ER respectively on VLB metabolism. Moreover, VLB significantly inhibited the biotransformation of VDS (Ki=3.8 µM) and vice versa. Furthermore, using a well characterized human hepatic microsome library, the biotransformations of VDS and VLB were significantly correlated with the erythromycin N-demethylase activity (r_{VDS}=0.80, r_{VLB}=0.83, $p<0.01$). Knowing that erythromycin N-demethylation is mediated by the human microsomal P-450 IIIA isozyme, our results strongly suggest the involvement of the P-450 IIIA isozyme in the hepatic biotransformations of these anticancer agents. (Supported by l'ARC)

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COMPARISON AMONG THE THREE METHODS OF DETERMINIG DRUG-PROTEIN BINDING

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The binding of indomethacin to protein was determined by three method: equilibrium dialy - sis, gel filtration and ultrafiltration.The results show that the ultrafiltration is faster and more simple then the others in performing and interfered by a few factors.Micro ultrafiltration-tube absobs very little sample . It is convenient that the ultrafiltration is used in clinical study.

THE USE OF EARLOBE BLOOD SAMPLE IN STUDIES ON THE PHARMACOKINETICS OF DIGOXIN

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Single doses of digoxin have been given to 5 healthy adult men and 4 rabbits.After administration drug plasma concentrations of men's earlobe and rabbit's ear-edge and same time vein were detected by radioimmunoassay (RIA). The results have indicated that both men's earlobe plasma are accurate reflection of the digoxin's change in body as time goes on. The data can be fitted by 2 compartment-model.Mean values of pharmacokinetics parameter are $t_{\frac{1}{2}}\alpha$ =2.108, $t_{\frac{1}{2}}\beta$ =38.250, $t_{\frac{1}{2}}ka$=0.687 hr, k_{21}=0.127 k_{12} =0.173, k_{10} =0.047/hr(5 human); $t_{\frac{1}{2}}\alpha$ =2.31(2.47)*, $t_{\frac{1}{2}}\beta$ =45.33(50.02), $t_{\frac{1}{2}}ka$=0.54(0.59)hr, k_{21} = 0.11(0.10), k_{12} =0.16(0.15), k_{10} =0.04(0.04)/hr, (4rabbits).

*Not:The vein plasma parameters are round brackts.

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STUDIES ON THE PHARMACOKINETICS WITH EARLOBE BLOOD SAMPLE

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The pharmacokinetics of Gentamycin(G), Streptomycin(S), Amikacin(A), Kanamycin(K), Digoxin(D) Phenytoin(P)and Methotrexat(M) has been studying since 1983. The results showed the earlobe blood drug concentration can accurately reflects the proceses of the drug quantitative change in body, there is a significant correlation between drug concentration of earlobe blood and synchronal venous blood (confidence:99%) . This suggests that the pharmacokinetics can be studied with earlobe blood sample if micro earlobe blood sample can be seperated and measured.

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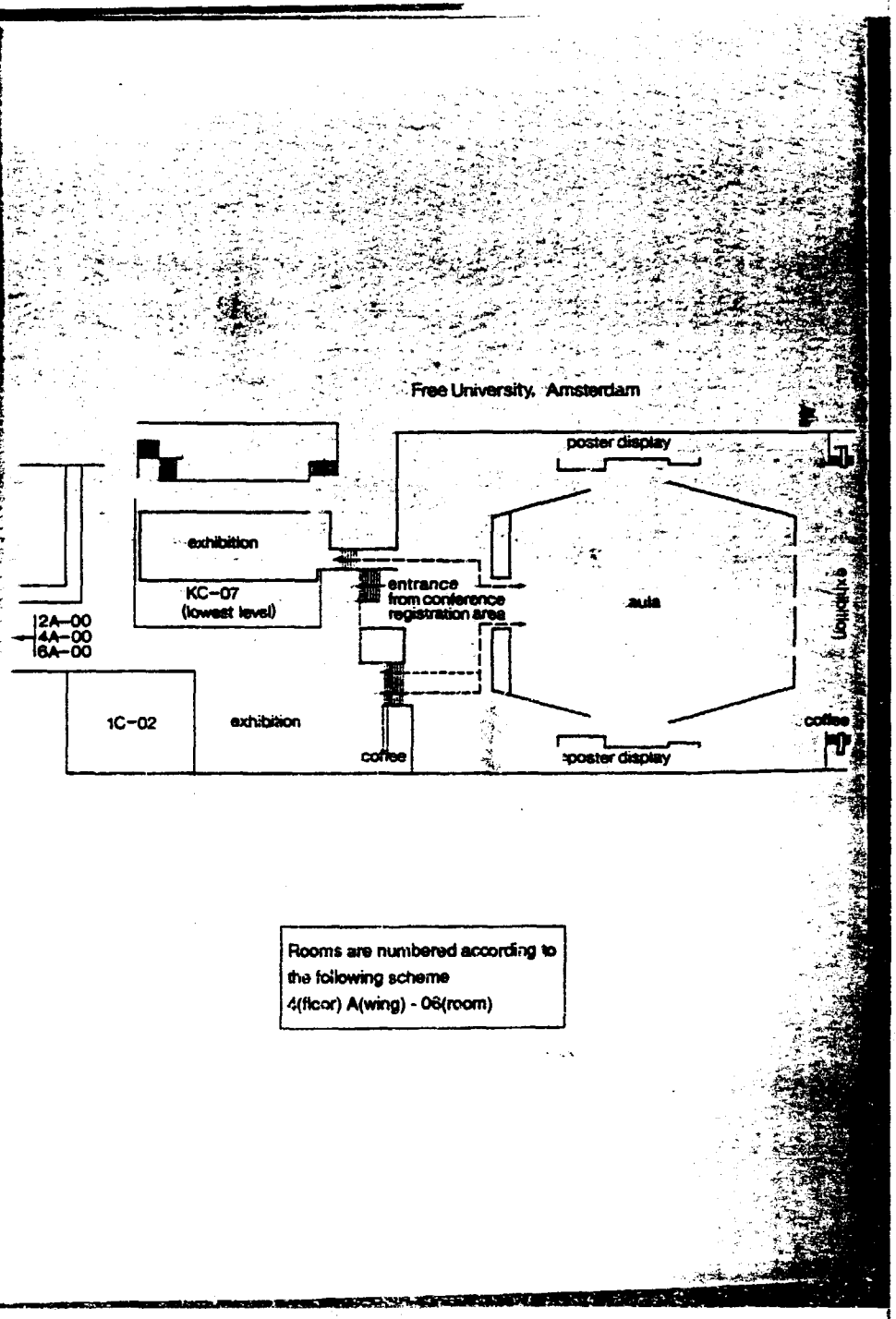

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